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**Seed number and 100-seed weight of pearl millet (*Pennisetum glaucum* L.) respond differently to low soil moisture in genotypes contrasting for drought tolerance**

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**Abstract**

Water stress after flowering, one of the major factors limiting yields of pearl millet, affects both seed setting and grain filling, and is a consequence of more/less water used prior to anthesis. However, whether genotypes have different sensitivities for seed setting and filling under drought, if exposed to similar stress intensity, is unclear. Experiments were conducted in two pairs of pearl millet genotypes, i.e. PRLT2/89-33 and H77/833-2, 863B and 841B, contrasting for terminal drought tolerance, and two genotypes, ICMR 01046 and ICMR 01029 (IL-QTLs) introgressed with a terminal drought tolerance QTL from PRLT2/89-33 into H77/833-2. Total seed weight, panicle number, 100-seed weight, seed number, and stover biomass were measured at different soil moistures, and throughout-grain filling. Sensitive H77/833-2 had higher seed number and yield under well watered (WW) conditions than in PRLT2/89-33 and IL-QTLs. Upon increases in water stress intensity, H77/833-2 suffered losses mostly in stover biomass (45%) and seed number (60%) at 0.3 FTSW whereas the biomass and seed number of PRLT2/89-33 decreased little (20% and 25%). The 100-seed weight of H77/833-2 decreased only 20% under stress. Tolerant 863B also maintained a higher seed

number and biomass under water stress than 841B. Seed growth duration in PRLT2/89-33 and IL-QTLs was similar to that of H77/833-2 under WW conditions but lasted longer than in H77/833-2 under water stress (WS). Similarly, seed growth of 863B was longer than 841B under WS. It is concluded that the higher seed yield of tolerant parents PRLT2/89-33 and 863B, and of IL-QTLs under WS was explained by the retention of a higher number of seeds than in sensitive lines, while the decrease in the 100-seed weight was proportionally less than the decrease in seed number. Phenotype with lesser number and larger size of panicles and larger grain size, like genotypes PRLT2/89-33 and 863B, withstood post-anthesis water stress better. IL-QTL inherited part of these characteristics, indicating a role for the terminal drought QTL in maintaining larger seed number and higher 100-seed weight. The continuous stover biomass increase under WW in H77/833-2, due to tillering, might indicate that tiller growth and grains are in competition for resources after anthesis and this may relate to the relatively shorter grain filling period.

**Key words:** Post flowering water stress, carbohydrates, transpiration, biomass, yield components

## Introduction

Pearl millet is widely grown in the arid zone of northwestern India and also in the Sahelian zone where there is no alternative. Drought stress is a regular feature in these environments, occurring at unpredictable time and intensity (Sharma and Pareek, 1993; van Oosterom et al., 1996), but being most common during grain filling. Successful grain filling is therefore one of the criteria in selecting the genotypes for improved adaptation to stress. Farmers preferentially grow high tillering landraces particularly when drought stress is highly unpredictable as in the case of arid areas of western Rajasthan (Van Oosterom et al., 1996). High tillering genotypes are associated with small sized panicles and low individual grain mass, which can be further decreased if the grain filling ability is impaired by water stress. However, genotypes with this type of development pattern are better able to cope with unpredictable stress because of their better capacity to compensate for the failure of the main panicle than low tillering large sized panicles (Bidinger and Hash, 2004). By contrast, genotypes with large grain size and low tillering have been widely adopted (Kelley et al., 1996; van Oosterom et al., 1996) in the wetter eastern areas of Rajasthan where pre-flowering drought stress is unlikely to occur but post-anthesis drought is predominant. The question of grain size under stress conditions is also important to address for grain quality since larger

seeds have a higher flour yield (Rooney and McDonough, 1987) and a stress effect on grain size could decrease flour yields. One of the unanswered questions is whether these different grain types are differently affected by water stress. In pearl millet grain yield is highly correlated with grain number (Bidinger and Raju, 2000). Large grain number correlates with small individual grain mass and short grain filling periods, and is an important adaptive feature of pearl millet to the arid climates (DeWet et al., 1992). In contrast, large grain size is a highly preferred characteristic according to farmer survey, allowing higher market price (Phul and Athwal, 1969, IARI report in magazine- National Herald 2006). Large grain mass also confers faster rates of seedling emergence, faster initial seedling and early crop growth (Lawan et al., 1985, Siband et al., 1978, Chhina and Phul, 1982), improved processing quality of the grain, easy decortications, and better flour yield with both commercial milling and hand-pounding milling methods (Rooney and McDonough, 1987). Whether different grain types are differently affected by terminal stress is not known in pearl millet.

A major drought QTL on linkage group two (LG2) explaining 23% of variation in grain yield under severe drought environments was identified (Yadav et al., 2002; Yadav et al. 2004; Bidinger et al 2007), and accounted for a better seed set and a better grain filling. This, in turn, was explained by a conservative water use when water was non-limiting, which made more water remains available for grain filling (Kholová et al., 2010a, 2010b; Vadez et al., 2013). Since the QTL is responsible for differences in grain filling, another possible explanation for the difference between tolerant and sensitive lines could be differences in the soil moisture thresholds where grain filling stops. Such information is not available in pearl millet and the existence of lines (ILs) introgressed with a major terminal drought tolerance QTL allows this exploration. Here, we address this question by following grain filling in different soil moisture conditions. Our hypothesis is that grain filling may stop at different levels of soil moisture in different genotypes which could explain part of the grain yield differences under terminal drought conditions.

The first objective of this study was to evaluate whether the response of yield components to drought stress differed between tolerant and sensitive genotypes, which was done by measuring grain yield, grain number, grain size, and stover biomass under different levels of soil moisture. The second objective was to assess how these different components evolved during the grain filling period in different genotypes, and this was done by sequential harvests during grain filling and until maturity. The work was carried out using contrasting

genotypes, including ILs containing a terminal drought tolerance QTL in order to assess whether mechanisms related to differences in seed filling are underlying this QTL.

## Materials and methods:

### Plant material

Parental genotypes: Pearl millet genotypes differing in terminal drought tolerance were selected. Two pairs of parents PRLT2/89-33 (tolerant) and H77/833-2 (sensitive), ICMB 863-P2 (tolerant, then referred to as 863B) and 841B-P3 (sensitive, then referred to as 841B) selected for the study contrasted for seed yield under terminal drought conditions based on previous experiments (Yadav et al., 2002; Serraj et al., 2005). Both pairs of parental lines were tested in that study because a terminal drought tolerance QTL was identified in both derived mapping populations. Tolerance/sensitivity was assessed using testcross hybrids of these parental inbred lines, using 843A and H77/833-2A as a male sterile tester for each pair respectively. PRLT2/89-33 is a low tillering, large panicle experimental line (Andrews and Anand Kumar, 1996) and H77/833-2 is a high tillering line with small panicles (Kapoor et al., 1989). More details describing the two parental pairs can be found in Kholová et al., (2010a).

Introgression lines (IL-QTLs): QTL introgression lines were developed in the background of sensitive parent H77/833-2 (recurrent parent) by introgressing the QTL from drought tolerant donor parent PRLT2/89-33 (QTL identified on LG2 by Yadav et al., 2002; Yadav et al. 2004; Bidinger et al., 2007) and the resulting F1 was backcrossed to the recurrent parent H77/833-2 for four generations (more details in Kholová et al., 2010a). IL-QTLs in H77/833-2 background (ICMR01029 and ICMR01046) were also tested as test-cross hybrids using 843A as a male sterile tester.

### Assessment of grain filling and seed number under different levels of soil moisture

Experiment 1 (Exp.1) was designed to assess the response of transpiration and of agronomic characteristics, including grain size and grain number, to different levels of moisture stress, in two parental pairs; i.e. PRLT 2/89-33 and H77/833-2, 863B and 841B . Plants were grown in pots filled with 9.5 kg of a mixture of Alfisol, sand, and manure (5:2:1) under glass house conditions with 17.6°C min and 35.5°C max temperature and 40-75% relative humidity (RH). Growth in the pots was very satisfactory and plant height was similar to the field conditions. Although small pot size could have affected the root/shoot ratio, these growth conditions

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131 were unlikely to have affected genotypic differences in response to drought. Five water  
132 treatments were used: One well-watered control and four water stress treatments in which the  
133 soil moisture content was re-adjusted daily to a constant value of 50%, 40%, 30%, and 20%  
134 of the fraction of transpirable soil water (i.e. 0.5, 0.4, 0.3, 0.2 FTSW) by deducting 890,  
135 1070, 1250 and 1420g of water, respectively, from the pots maintained at field capacity. This  
136 was based on previous experiments in which it was shown that, when exposed to a  
137 progressive water stress: (i) genotypes did not vary in the amount of water they could extract  
138 for transpiration from a given soil weight; (ii) there was approximately 180g of transpirable  
139 water per kg of soil. These FTSW values reflected an average for the entire pot and it does  
140 not exclude the possibility that, at re-watering, the top part of the pot would have been wet  
141 while the remaining part of the pot would have been dry. We believe this was not an issue for  
142 the plant response. First, this would be a similar situation in nature. Second, the most limiting  
143 factor for the plant was the limited amount of water that was received every day and this was  
144 relatively similar for each genotype. The experimental design was a complete block design  
145 with water treatment as main block and genotypes as sub-factor in each main block and  
146 randomized five times.

147       Plants were grown under fully irrigated conditions until panicle emergence. As soon  
148 as panicles were emerged all the pots were watered and allowed to drain overnight to reach  
149 field capacity. The following morning, pots were wrapped with plastic bags around the base  
150 of the stem to cease soil evaporation and pots were subsequently weighed. Pots from all  
151 treatments were maintained under well-watered (WW) conditions by daily re-watering the  
152 pots up to 80% field capacity (0.8 FTSW) until flowering. Water stress treatments were  
153 imposed from flowering time onwards by gradually decreasing the water level to 0.5, 0.4, 0.3,  
154 and 0.2 FTSW. All genotypes flowered within about two to three days from one another, so  
155 that there was only a minimum time difference with the time when water stress was initiated.  
156 The FTSW represents how much water is available for transpiration in the pot, as a  
157 proportion of what is available at field capacity (1, or 100%). In order to impose a gradual  
158 stress, the desired soil moisture levels were reached only 4-5 days after flowering. The  
159 purpose of this was also to ensure that reproduction would take place under WW conditions  
160 and before the stress levels were imposed. The pot weights were maintained at these set  
161 levels of FTSW until maturity (between 30 and 38 days after flowering), by daily weighing  
162 and re-watering to set target pot weight. Harvested plants were oven-dried in a forced-air  
163 oven at 70°C for three days. Stover biomass, panicle number, total seed weight, seed number,  
164 and 100- seed weight were then measured.



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**166 Dynamics of grain filling**

167 Experiment 2 and 3 (Exp.2 and Exp.3) followed up how grain yield, grain number, grain size,  
168 stover biomass, and transpiration evolved during the grain filling period in plants exposed to  
169 two water regimes: (i) a well-watered control (WW); (ii) a soil moisture content of 0.3 FTSW  
170 (with 30% moisture) from four days after flowering and until maturity. Exp.2 included ICMR  
171 01029 and ICMR 01046, PRLT2/89-33 and H77/833-2. Exp.3 included 863B and 841B. The  
172 transpiration values, obtained from daily weighing, were normalized against control plants to  
173 get normalized transpiration ratio. For that, the transpiration value of each replicate was  
174 divided by the average transpiration of WW plant to get a transpiration ratio. A second  
175 normalization was carried out to take care of plant to plant variation in size, by dividing the  
176 transpiration ratio by the mean transpiration ratio value of the first three days of the  
177 experiment, before the occurrence of any stress. Sequential harvests were carried out at 10,  
178 20, and 30 days after flowering, the last harvest corresponding to maturity. The experimental  
179 design was a complete block design with the three sequential harvests as main blocks, water  
180 regimes as sub-blocks, and genotypes as sub-factor in each main sub-block and randomized  
181 five times. Plants were grown under same conditions. Treatment imposition and harvest  
182 procedures followed those of Exp.1.

183 Extraction and determination of total carbohydrates was done from the penultimate  
184 internodes (below the peduncle) harvested at maturity, by grinding 200mg of fresh tissue  
185 twice with 70% ethanol at 90°C. These ethanol extracts were pooled, centrifuged at 10,000 g  
186 for 10min and the supernatant was taken for estimation. Total sugars were estimated by  
187 Dubois et al. (1956) method using phenol and sulphuric acid.

188

**189 Statistical analysis:**

190 One way ANOVA was carried out for genotypic differences within the treatment. ANOVA  
191 was done with the statistical program package CoStat version 6.204 (Cohort Software,  
192 Monterey, CA, USA). Grouping of the genotypes in between the treatments was done using  
193 Duncan's multiple range tests through the statistical program SAS version 9.2 to compare the  
194 treatment effect from Exp.1. For Exp.2 and Exp.3, Duncan's multiple range tests through  
195 SAS version 9.2 was used to compare the genotypes at different times of harvests separately  
196 for control and stress during the grain filling period. ANOVA was carried out for genotypic  
197 differences in carbohydrates separately under WW and water stress.



**Results:**

**Grain yield response to varying water stress treatments**

Seed yield decreased significantly at 0.4 FTSW compared to the WW treatment, and then further decreased significantly at 0.3 and 0.2 FTSW in both the parental pairs ( $P < 0.0001$  Fig. 1a and 1b, Exp.1). However, genotypic differences in total seed weight were observed under WW conditions and 0.3 FTSW in both pairs. There were significant genotype-by-treatment interactions for the seed yield ( $P < 0.02$ ), so that under WW conditions, H77/833-2 had significantly higher total seed weight than PRLT2/89-33 ( $P < 0.1$ ), whereas at 0.3 and 0.2 FTSW, PRLT2/89-33 had higher total seed weight than H77/833-2 ( $P < 0.1$  Fig. 1a, Exp.1). With the parental pair 863B and 841B (Exp.1, Fig. 1b), genotype 863B (tolerant) had significantly higher total seed weight than 841B (sensitive) under WW conditions ( $P < 0.01$ ) and water stress treatments 0.4 and 0.3 FTSW ( $P < 0.1$ ).

The evolution of seed weight over time after flowering at 0.3 FTSW and under WW conditions was followed in Exp.2 (Fig. 1c). Under WW conditions, the seed weight of H77/833-2 significantly increased in all sequential harvests ( $P < 0.0001$ ), with the highest seed yield ( $P < 0.05$ ) at maturity, whereas in PRLT2/89-33, ICMR 01046, and ICMHR 01029 the total seed weight did not increase significantly beyond 20 days after flowering. Under WS conditions (0.3 FTSW), the increase in seed weight was somewhat slower, with a gradual increase in total seed weight. All genotypes attained their maximum seed weight at 20 days after flowering except ICMR 01046 that reached its highest total seed weight at 30 days after flowering (Fig. 1c). PRLT2/89-33, ICMR 01029 and ICMR 01046 had significantly higher total seed weight than H77/833-2 at maturity ( $P < 0.05$ ), in agreement with Exp. 1. Under WW conditions in the parental pair 863B and 841B (Exp.3, Fig. 1d), the total seed weight increased significantly until the last harvest ( $P < 0.05$ ). Under WS conditions, both the parental lines showed maximum seed weight at 20 days after flowering (Fig. 1d). There, 863B (tolerant) had significantly higher seed weight than 841B (sensitive) under WW and WS conditions in agreement with Exp.1 ( $P < 0.05$ ).

**Panicle number, seed number and 100-seed weight response to water stress**

Water stress strongly decreased panicle number, especially in the high tillering types. Indeed, panicle number decreased at treatment 0.3 FTSW ( $P < 0.0001$ , Exp.1, Fig.2a) and these changes were driven by a sharp decrease in panicle number of high tillering H77/833-2 (sensitive parent, ( $P < 0.01$ ), reflecting significant genotype-by-treatment interaction ( $P < 0.05$ ). In the parental pair 863B and 841B (Exp.1, Fig.2b) significant treatment differences were observed at 0.4 FTSW with no further decrease at more severe treatment ( $P < 0.0001$ ). Similar to the above parental pair 841B (sensitive parent) had significantly higher number of panicles in all the treatment except at 0.3 FTSW ( $P < 0.01$ ).

The seed number of the parental pair PRLT2/89-33 and H77/833-2 decreased significantly at 0.4 FTSW although this decrease affected only H77/833-2 compared to WW conditions. The seed number of the parental pair then further decreased at 0.2 FTSW ( $P < 0.0001$ ) and seed number decreased in both genotypes compared to 0.4 FTSW (Exp.1, Fig. 3a). The genotype-by-treatment interaction for seed number was also highly significant ( $P < 0.001$ ). H77/833-2 (sensitive parent) had significantly higher seed number than PRLT2/89-33 (tolerant parent) under WW, 0.5 FTSW, and 0.4 FTSW ( $P < 0.01$ ) but both genotypes had similar seed number at 0.3 and 0.2 FTSW. At these FTSW levels, the seed number of H77/833-2 was 65% less than the WW conditions. In the parental pair 863B and 841B (Exp.1, Fig. 3b), the seed number decreased significantly at 0.4 FTSW compared to WW conditions, with a further significant decrease at 0.3 and then 0.2 FTSW ( $P < 0.0001$ ). Genotypes 863B and 841B had similar seed number at all FSTW levels except 0.3 ( $P < 0.05$ ).

In Exp.2 (Fig. 3c) the evolution in the seed number over time after flowering was observed. Under WW conditions the seed number remained similar in PRLT2/89-33 throughout the grain filling period. By contrast, the seed number increased throughout the seed filling period in the other genotypes. In ICMR 01029 the maximum seed number was observed at 20 days after flowering whereas ICMR 01046 and H77/833-2 reached the highest seed number at 30 days. This was likely related to the development of reproductive tillers in these three high tillering types. Under WS conditions the seed number of PRLT2/89-33 and ICMR 01046 remained similar across the different harvests. By contrast, the seed number increased in H77/833-2 and ICMR 01029 and the highest seed number was reached at 20 days and 30 after flowering respectively. However, even at its highest seed number (20 days after flowering) the seed number of H77/833-2 had decreased drastically compared to the WW treatment while it did decrease relatively less in the other genotypes, in agreement with Exp.1. In the parental pair 863B and 841B under WW conditions the maximum seed number

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262 was reached at the last harvest i.e., 30 days after flowering in both 863B (tolerant) and 841B  
263 (sensitive) (Exp.3, Fig. 3d). Under water stress, there was no further significant increase in  
264 seed number in both genotypes beyond 20 days after flowering.

265         The 100-seed weight started to decrease at lower soil moisture levels than biomass  
266 and seed yield. In the PRLT2/89-33 and H77/833-2 pair (Exp.1, Fig. 4a), there was no  
267 significant treatment effect on the 100-seed weight until FTSW was down to 0.2. The  
268 genotype-by-treatment interaction for the 100-seed weight was also highly significant ( $P$   
269  $<0.004$ ). The 100-seed weight decreased gradually in PRLT2/89-33 from 0.5 to 0.3 FTSW,  
270 and the 100-seed weight of PRLT2/89-33 was about 23% lower at 0.3 FTSW than under WW  
271 conditions, with a rapid drop between 0.3 and 0.2 FTSW. By contrast, in H77/833-2 there  
272 was no change in the 100-seed weight between the WW control and 0.3 FTSW, but there was  
273 a rapid drop at 0.2 FTSW. Therefore, PRLT2/89-33 (tolerant parent) had significantly higher  
274 100-seed weight than H77/833-2 (sensitive) until FTSW was down to 0.3 ( $P <0.001$  at WW  
275 and  $P <0.05$  at 0.3 FTSW) but both the genotypes had similar low 100-seed weights at 0.2  
276 FTSW. In the parental pair 863B and 841B the 100-seed weight decreased significantly at 0.3  
277 FTSW compared to the WW treatment, and then further decreased at 0.2 FTSW ( $P <0.0001$ ,  
278 Exp.1, Fig. 4b). Genotypic differences were significant until FTSW was down to 0.4 with  
279 863B parent having a significantly higher seed weight than 841B ( $P <0.01$ ). At 0.3 and 0.2  
280 FTSW the 100-seed weight was not significantly different between the two genotypes.

281         The evolution of the 100-seed weight was followed at 0.3 FTSW and under WW  
282 conditions in the post-flowering and grain filling period. Under WW conditions all the  
283 genotypes attained the maximum 100-seed weight (used as a proxy for seed filling) at 20  
284 days after flowering and there was no further significant increase at 30 days after flowering.  
285 Under water stress conditions the increase in 100-seed weight was more gradual. For instance  
286 PRLT2/89-33, ICMR 01046, and ICMR 01029 reached their maximum 100-seed weight at  
287 30 days after flowering. By contrast, the 100-seed weight did not change in H77/833-2 across  
288 the different harvests (Exp. 2; Fig. 4c). Unlike Exp.1, the 100-seed weight of H77/833-2  
289 decreased at 0.3 FTSW compared to the WW conditions. However, in both experiments it  
290 was the large seed number decreased that affected the seed yield of H77/833-2. In the  
291 parental pair 863B and 841B under both WW and WS conditions both genotypes 863B and  
292 841B reached their maximum 100-seed weight at 20 days after flowering (Exp. 3; Fig. 4d).

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## Stover biomass, transpiration and total soluble sugars response to water stress

Stover biomass decreased gradually with increase in the water stress treatment (Exp.1, Fig. 5a). In the parental pair PRLT2/89-33 and H77/833-2 it was not until 0.3 FTSW that a significant decrease in biomass was observed ( $P < 0.0001$ ). However, the genotype-by-treatment interaction for stover biomass was not significant. Within treatment, significantly higher biomass was observed in PRLT2/89-33 (tolerant) than H77/833-2 (susceptible) at 0.3 FTSW only ( $P < 0.05$ , Fig. 5a). In the parental pair 863B and 841B (Exp. 1, Fig. 5b) stover biomass decreased significantly compared to the WW control at 0.3 and 0.2 FTSW ( $P < 0.0001$ ). Within treatments 863B (tolerant) had significantly higher biomass than 841B (sensitive) at 0.3 and 0.2 FTSW treatments ( $P < 0.05$ , Fig. 5b).

The evolution of stover biomass over time after flowering was followed at 0.3 FTSW and under WW conditions at different timings after flowering. Under WW conditions there was no increase in the vegetative biomass across the three different harvests in PRLT2/89-33 (Exp. 2; Fig. 5c). By contrast, in the other three lines stover biomass increased between flowering and maturity. In H77/833-2 the highest accumulation of stover biomass was observed at the last harvest (30 days after flowering) while in ICMR 01046 and ICMR 01029, there was no significant increase in stover biomass beyond 20 days after flowering (Fig. 5c). Under WS conditions none of the genotypes showed any significant increase in stover biomass after flowering (Fig. 5c) but at maturity PRLT2/89-33 (tolerant) had significantly higher biomass than H77/833-2 (sensitive), in agreement with Exp.1 ( $P < 0.05$ ). In the parental pair 841B and 863B there was no increase in the stover biomass after flowering irrespective of the time of harvest in any of the water treatment (Fig. 5d). Under WS conditions, 863B had significantly higher biomass than 841B in agreement with Exp.1 ( $P < 0.05$ ).

The transpiration of the WW plants was higher than in the different FTSW treatment although the difference with the 0.5 FTSW was small. Within the WW (well watered) and the different water stress treatments (0.5, 0.4, 0.3, 0.2 FTSW) of Experiment 1 (Fig. 6a) there were only slight differences between tolerant and sensitive genotypes. However, after normalizing the transpiration data for each individual genotype tolerant parent PRLT2/89-33 had lower NTR (normalized transpiration ratio) than the sensitive parent H77/833-2 at 0.3 FTSW (Fig. 6b). Similar results were observed in the case of 863B and 841B parental pair where the difference in transpiration showed no clear trend (Fig. 6c) but where 863B (tolerant parent) had lower NTR than 841B (sensitive parent) at 0.3 and 0.5 FTSW (Fig. 6d). In Exp.2,

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transpiration (Supplementary Fig. 1a) was similar in PRLT 2/89-33, H77/833-2, ICMR 01046, and ICMR 01029 but the NTR of tolerant parent PRLT2/89-33 and ICMR 01029 was lower than the sensitive parent H77/833-2 and ICMR 01046 (Supplementary Fig. 1b). Similar results were found in Exp.3, where transpiration (Supplementary Fig. 1c) was similar in both the genotypes but NTR (Supplementary Fig. 1d) of tolerant parent 863B was lower than sensitive parent 841B in the middle of the grain filling period. This trend of lower NTR was also observed in Experiment 1 (Fig. 6d) although it was not significant then.

Under WW conditions and water stress in Experiment 2 and 3 (Fig. 7), soluble sugars differed significantly among the genotypes. Soluble sugars decreased dramatically under WS in PRLT2/89-33, ICMR 01029 and 863B, whereas it did not decrease in H77/833-2, ICMR 01046 and 841B. The soluble sugars were expressed per unit of fresh weight. We do not expect the stem relative water content to vary much between genotypes under WS at 30 days after flowering (i.e. their absolute transpiration was similar across genotypes), so that genotypic differences under WS conditions would likely not change if data were expressed per unit of dry weight.

**Discussion**

**Grain yield decrease under drought was due to a greater effect on seed number than on seed size**

The higher seed yield of sensitive parent H77/833-2 under WW conditions was explained by its higher number of productive tillers (Fig. 2a) which would also explain why seed yield increased between the harvest at 20 days after flowering and the harvest at maturity (Fig. 1c). Under WS conditions the large seed yield decrease of H77/833-2 was then mostly due to a decrease in seed number, which was in part explained by a decreased number of panicles whereas in PRLT2/89-33 the yield decrease was related to a decrease in the 100-seed weight. However, the reduction of 100-seed weight in PRLT2/89-33 was proportionally less (23% at 0.3 FTSW compared to the WW conditions, Fig. 4a) than the reduction of seed number in H77/833-2 (about 65% lower at 0.3 FTSW than under the WW conditions, Fig. 3a). This explained the lower seed yield of H77/833-2 than PRLT2/89-33 under WS conditions. Therefore, the lower seed yield in H77/833-2 was due to combined effect of a reduction in panicle number (productive tillers) and a drastic reduction in seed number. The reduced seed number might itself result from a combination of decreased number of panicles bearing seeds



and abortion of some of the grain after flowering, although we don't have sufficient data to fully conclude on this. Therefore, future work should look at each individual tiller to assess the decrease in seed number and seed size in each of these, in relation to the timing of flowering of these tillers. An interesting insight would then be to compare sink strength in each tiller and carbohydrate content. In the 841B and 863B pair the number of seeds also decreased relatively more in 841B than in 863B at 0.3 FTSW and decrease yield more in 841B. These results are in agreement with Blum et al. (1990) who suggested that yield reduction under water stress at later stages was mainly due to the number of grains per spike. These data agree with earlier work in pearl millet (eg Bidinger et al., 1987; 2004). Similar results were reported by Izanloo et al. (2008) in wheat who reported that water stress reduced yield through tiller abortion and lower grain number per spike. Interestingly, similar observations have been made in legumes, i.e. in bean (Szilagyi., 2003), and chickpea (Zaman-Allah et al., 2011), where the reduction in seed yield under water stress was due to a decrease in pod number per plant and in seed number per pod, but not to a reduction in the 100-seed weight.

The higher seed yield of PRLT2/89-33 under WS conditions was then due to its capacity to retain seed number relatively unchanged at 0.3 FTSW (about 75% of that under WW conditions) and to limit the reduction in seed size. This may be related to the fact that the increase in grain size is limited in high tillering genotype H77/833-2 either due to genetically maximum grain size or inadequate availability of assimilates for grain filling. Response of stronger sink towards higher yields has been reported in barley and wheat (Voltas et al., 1997; Cartelle et al., 2006). Similarly, the low-tillering large seeded genotype PRLT2/89-33 have greater ability to adjust grain number and individual grain mass thus affecting panicle productivity (Bidinger and Raju, 2000). These genotypes have been bred for higher yield through maintaining higher individual grain mass.

### **Traits dynamics in parental and introgression lines**

Under well watered conditions, in PRLT2/89-33, there was no increase in vegetative biomass after flowering whereas the vegetative biomass of H77/833-2, but also that of introgressed lines having drought QTL of tolerant donor parent PRLT2/89-33, increased during grain filling. This increase in both vegetative biomass and in grain yield in the high tillering material was also possible because of the fairly wide spacing of the plants in the

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glasshouse (about 5 plant m<sup>-2</sup>). Under water stress (0.3 FTSW), a significant decrease in the vegetative biomass of high tillering H77/833-2 genotype was likely related to a decrease in the number of productive tillers, in part shown in Fig. 2a. Therefore, this led to a significantly lower yield than in PRLT2/89-33 and ILs. The advantage conferred by the introgression of the QTL over H77/833-2 was in two ways: (i) larger seed size than in H77/833-2 and the capacity to sustain seed filling well into the drought period, whereas the seed filling duration of H77/833-2 was short; (ii) having a seed number intermediate between the two parents and the capacity to retain a relatively high seed number under WS conditions. These experimental results are in agreement with previous findings (Serraj et al., 2005). Another advantage of the IL lines could have been in having larger panicle size than the recurrent parent, although we have no data to support this and only qualitative observations. Similar conclusions have also been drawn by Bolanos (1995) where superior yield of hybrids was mostly due to larger sink (larger ear weight and ear growth rate). PRLT2/89-33 and ILs had indeed a high grain filling ability under both WW and WS conditions, shown by their capacity to sustain seed filling until maturity, whereas the seed size of H77/833-2 was the same at each of the three harvests under WS conditions (Fig. 4c). ICMR 1046 maintained the 100-seed weight similar to drought tolerant PRLT2/89-33 parent under WS conditions. This provides us evidence that the donor QTL help in maintaining the grain filling ability. The seed number in ILs was also decreased less dramatically than in recurrent parent H77/833-2. Among the two introgression lines that are nearly isogenic, ICMR 01046 and ICMR 01029 were highly similar to PRLT2/89-33 parent for most traits. Thus, besides a large grain size with low grain number to keep up the yield under post flowering drought stress (Bidinger and Raju, 2000), as shown in the previous section, the added advantage of tolerant materials seems to be in the capacity to maintain grain filling for a longer period. This was not related to a higher water extraction capacity, but rather from having water saving mechanisms operating earlier in the crop cycle and making water available during the grain filling period (Kholova et al., 2010a; Vadez et al., 2013).

**Carbohydrate translocation and transpiration**

Synthesis, storage and mobilization of carbohydrates under water stress are essential processes for grain filling (Gupta et al., 2011). Reduction of assimilates under water stress that limits grain filling has been reported (Mahalakshmi et al., 1993). The percentage decrease in total soluble sugars was high in ICMR 01029 (52%) and tolerant parents



PRLT2/89-33 (24%), 863B (25%). However, there was no reduction under WS conditions in sensitive parents H77/833-2 and 841B (Fig.7). Therefore, we may interpret that part of the seed setting or seed filling failure was related to the inability to remobilize sugars from the stem in sensitive lines. The reasons for that are unknown but could be related to poor translocation or enzymatic activities, and further research would be needed in the mechanisms that regulate grain filling.

Transpiration, which was used as a simple proxy for photosynthesis, decreased with progressive exposure to water stress. The absolute transpiration values were not very different between genotypes at any of the treatment although relative to the control, the NTR of tolerant genotypes was below that of the sensitive parents. Interestingly, once the FTSW was set at each of the pre-determined levels, transpiration remained relatively constant and did not vary much between genotypes. Since the vegetative biomass did not increase in PRLT2/89-33 during grain filling under WW conditions, and it increased only slightly in ILs, the transpiration occurring during the grain filling period would have mostly supported the filling of grains. By contrast, in H77/833-2, the large increase in vegetative biomass during the grain filling period under WW implies that tiller growth continued well into the grain filling period and then contributed to grain yield, if water was available. Under water stress, although there was no significant increase in vegetative biomass, we may hypothesize that competition may have occurred between grain filling of the early tillers and the developing tillers, which may in part explain the failure of a number of grains, but also an inadequate carbon accumulation that limits tiller growth and then limits yield under WS. In the case of 863B, the higher transpiration efficiency of this line, reported earlier (Kholová et al., 2010b), might have also contributed to the higher vegetative biomass than in 841B under 0.3 FTSW, and therefore a higher yield. Therefore, higher yield of 863B parent was apparently due to its combined effort in keeping up the number of reproductive tillers and seed number in comparison to the sensitive parent 841B which had lower vegetative biomass (likely linked to lesser number of reproductive tillers, Fig.2b) and seed number under water stress (0.3 FTSW). This data is in support with previous results on these two lines (Yadav et al., 2004).

## Conclusion

Water stress affected the seed yield dramatically under terminal drought conditions, especially in sensitive genotypes (H77/833-2 and 841B). This was related to their yield

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architecture causing a decline in the number of productive panicles thereby effecting the seed number, ultimately the total seed weight. The seed number started showing the effect of water stress at levels of soil moisture where the 100-seed weight was still not affected and the magnitude of the decrease in seed number was higher than the magnitude of the decrease in the 100-seed weight. By contrast, the 100-seed weight was more affected in tolerant genotypes by water stress than in sensitive genotypes, even though all the genotypes had very severe 100-seed weight reductions at the most severe stress. Thus, retention of seed number and sustained seed filling under water stress resulted in higher yield in tolerant parents PRLT2/89-33 and 863B. However, these criteria may not be suited for unpredicted and pre-flowering water stress conditions. IL-QTL's followed the pattern of tolerant parent PRLT2/89-33 under water stress in their seed number and seed filling, which suggests that the terminal drought tolerance QTL may have some role to play in the maintenance of a higher number of seeds under water stress and a relatively higher grain size than recurrent parent H77/833-2.

**Acknowledgements**

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**Figure legends**

Figure 1. Total seed weight of two pairs of pearl millet test cross hybrids PRLT 2/89-33 and H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a well-watered control (WW) and four water stress treatments imposed by maintaining soil moisture at 0.5, 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with same letters on the x-axis were not significantly different. Star represented above the bar ( $\pm$ SE) indicates significant genotypic differences within the treatment ( $P < 0.1$ ). The total seed weight was also monitored at different days after flowering (DAF, c and d) in two pearl millet test cross hybrids of PRLT2/89-33 (tolerant), H77/833-2 (sensitive) and their NILs ICMR 01046, and ICMR 01029 (c) and in 863B and 841B (d) under well watered (WW) and water stress (WS) conditions (i.e. 0.3 FTSW). Values are means ( $\pm$ SE) of each genotype harvested at 10, 20, and 30 DAF. Values were compared across harvest time for each genotype and harvest time values for with same letters above the bar were not significantly different ( $P < 0.1$ ).

Figure 2. Panicle number of two pairs of pearl millet test cross hybrids: PRLT 2/89-33, and H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a well-watered control (WW) and four water stress treatments imposed by maintaining soil moisture at 0.5, 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with same letters on the x-axis were not significantly different. Star represented above the bar ( $\pm$ SE) indicates significant genotypic differences within treatment ( $P < 0.05$ ).

Figure 3. Seed number of two pairs of pearl millet test cross hybrids PRLT 2/89-33 and H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a well-watered control (WW) and four water stress treatments imposed by maintaining soil moisture at 0.5, 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with same letters on the x-axis were not significantly different. Star represented above the bar ( $\pm$ SE) indicates significant genotypic differences within the treatment ( $P < 0.01$ ). Seed number was also monitored at different days after flowering (DAF, c and d) in two pearl millet test cross hybrids of PRLT2/89-33 (tolerant), H77/833-2 (sensitive) and their NILs ICMR 01046, ICMR 01029 (c) and in 863B and 841B (d) under well watered (WW) and water stress (WS) conditions (0.3 FTSW). Values are means ( $\pm$ SE) of each genotype harvested at 10, 20, and 30 DAF. Values were compared across harvest time for each genotype ( $P < 0.05$ ) and harvest time values with same letters above the bar were not significantly different



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604 Figure 4. 100 seed weight of two pairs of pearl millet test cross hybrids PRLT 2/89-33 and  
605 H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a well-watered  
606 control (WW) and four water stress treatments imposed by maintaining soil moisture at 0.5,  
607 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with same letters on  
608 the x-axis were not significantly different. Star represented above the bar ( $\pm$ SE) indicates  
609 significant genotypic differences within the treatment ( $P < 0.05$ ). The 100 seed weight was  
610 also monitored at different days after flowering (DAF, c and d) in two pearl millet test cross  
611 hybrids of PRLT2/89-33 (tolerant), H77/833-2 (sensitive) and their NILs ICMR 01046,  
612 ICMR 01029 (c) and in 863B and 841B (d) under well watered (WW) and water stress (WS)  
613 conditions (0.3 FTSW). Values are means ( $\pm$ SE) of each genotype harvested at 10, 20, and 30  
614 DAF. Values were compared across harvest time for each genotype ( $P < 0.05$ ) and harvest  
615 time values with same letters above the bar were not significantly different.

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617 Figure 5: Stover biomass (Stem + leaves) of two pairs of pearl millet test cross hybrids PRLT  
618 2/89-33 and H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a  
619 well-watered control (WW) and four water stress treatments imposed by maintaining soil  
620 moisture at 0.5, 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with  
621 same letters on the x-axis were not significantly different. Star represented above the bar  
622 ( $\pm$ SE) indicates significant genotypic differences within treatment ( $P < 0.05$ ). The stover  
623 biomass (leaf and stem) was also monitored at different days after flowering (DAF, c and d)  
624 in two pearl millet test cross hybrids of PRLT2/89-33 (tolerant), H77/833-2 (sensitive) and  
625 their NILs ICMR 01046, ICMR 01029 (c) and in 863B and 841B (d) under well watered  
626 (WW) and water stress (WS) conditions i.e. 0.3 FTSW (fraction of transpirable soil water).  
627 Values are means ( $\pm$ SE) of each genotype harvested at 10, 20, and 30 DAF. Values were  
628 compared across harvest time for each genotype ( $P < 0.05$ ) and harvest time values with same  
629 letters above the bar were not significantly different.

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631 Figure 6: Daily transpiration (Exp.1, March 2009) of two pairs of pearl millet test cross  
632 hybrids PRLT 2/89-33 and H77/833-2 (a), 863B and 841B (c) exposed to different water  
633 regimes, i.e. a well-watered control (WW) and four water stress treatments imposed by  
634 maintaining soil moisture at 0.5, 0.4, 0.3, 0.2FTSW after flowering. NTR (normalized  
635 transpiration ratio) of these four parental lines PRLT 2/89-33 and H77/833-2 (b), 863B and  
636 841B (d) at 0.5 and 0.3 FTSW (fraction of transpirable soil moisture).



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Fig. 7: Total carbohydrates from the penultimate internodes of two pearl millet test cross hybrids of parental lines PRLT2/89-33 (tolerant), H77/833-2 (sensitive), their NILs ICMR 01046, ICMR 01029 ( Exp.2, April 2010 ) and 863B (tolerant), 841B(sensitive) parental lines harvested at maturity from well watered (WW) and water stress (WS) i.e. 0.3 FTSW (fraction of transpirable soil water).

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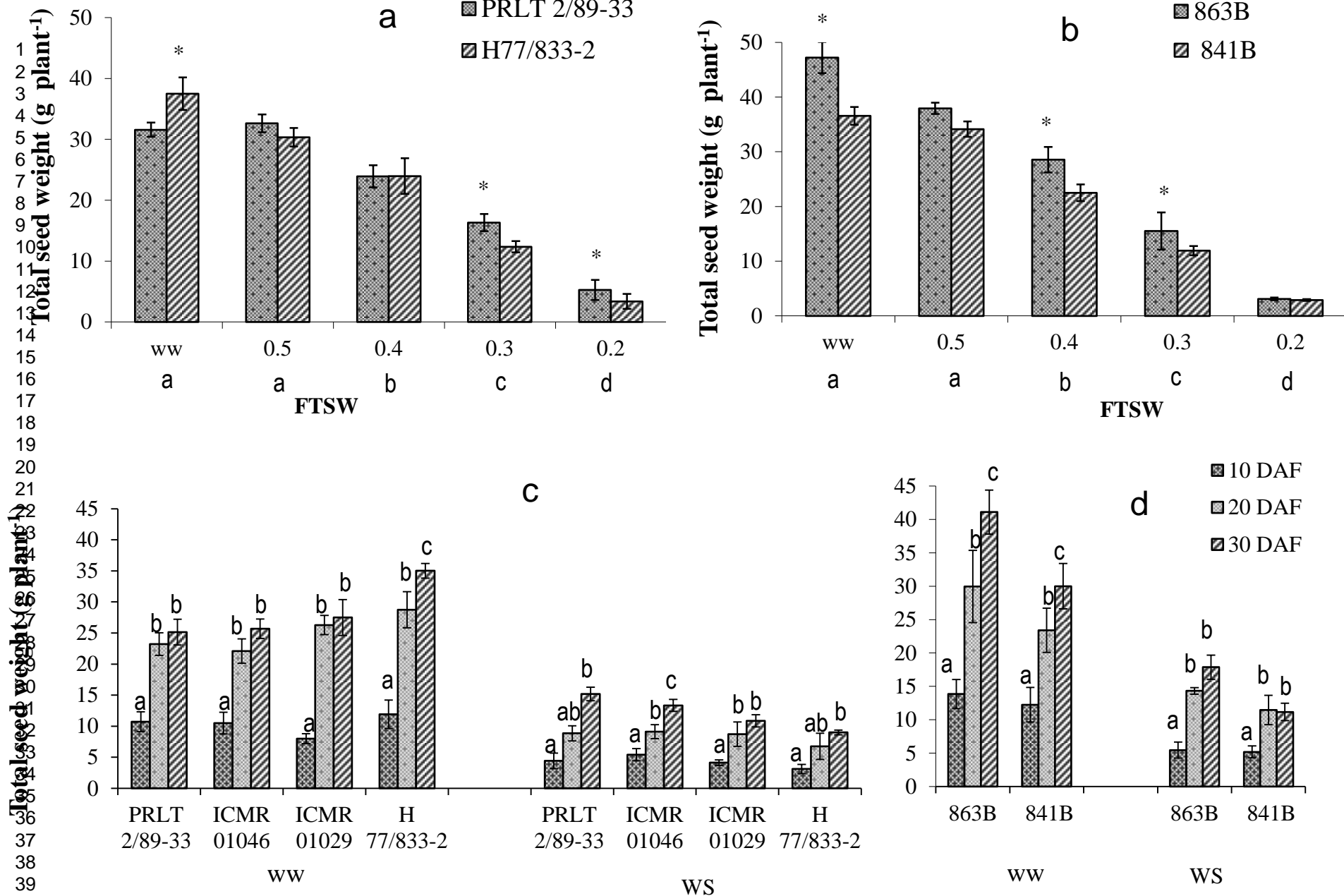


Figure 1

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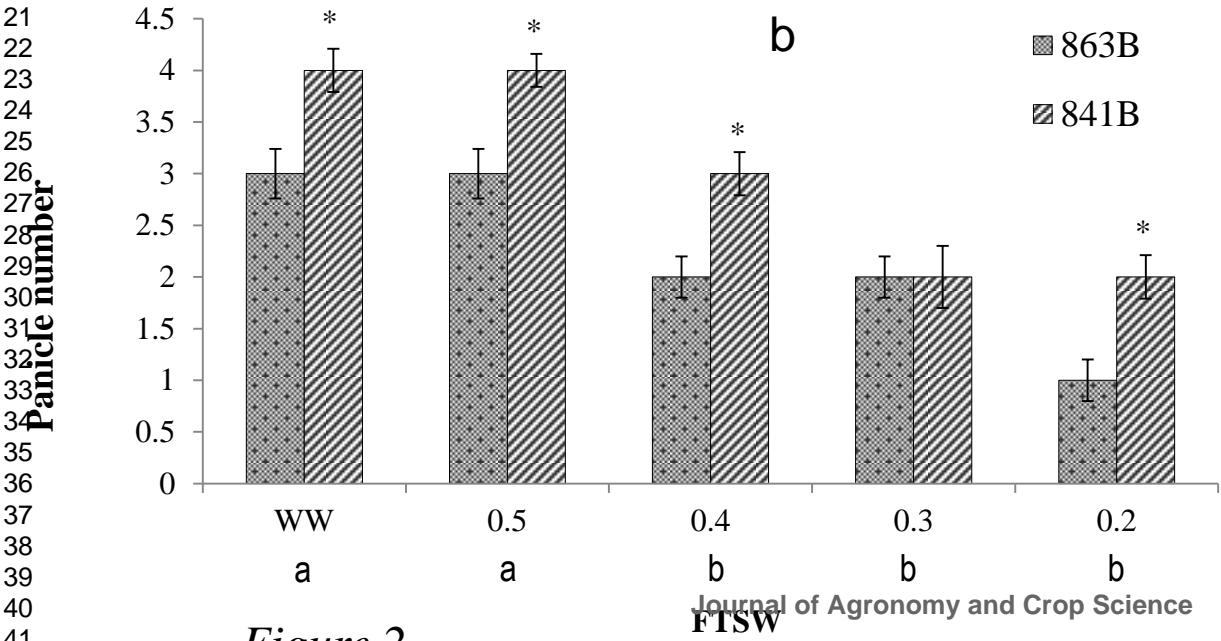
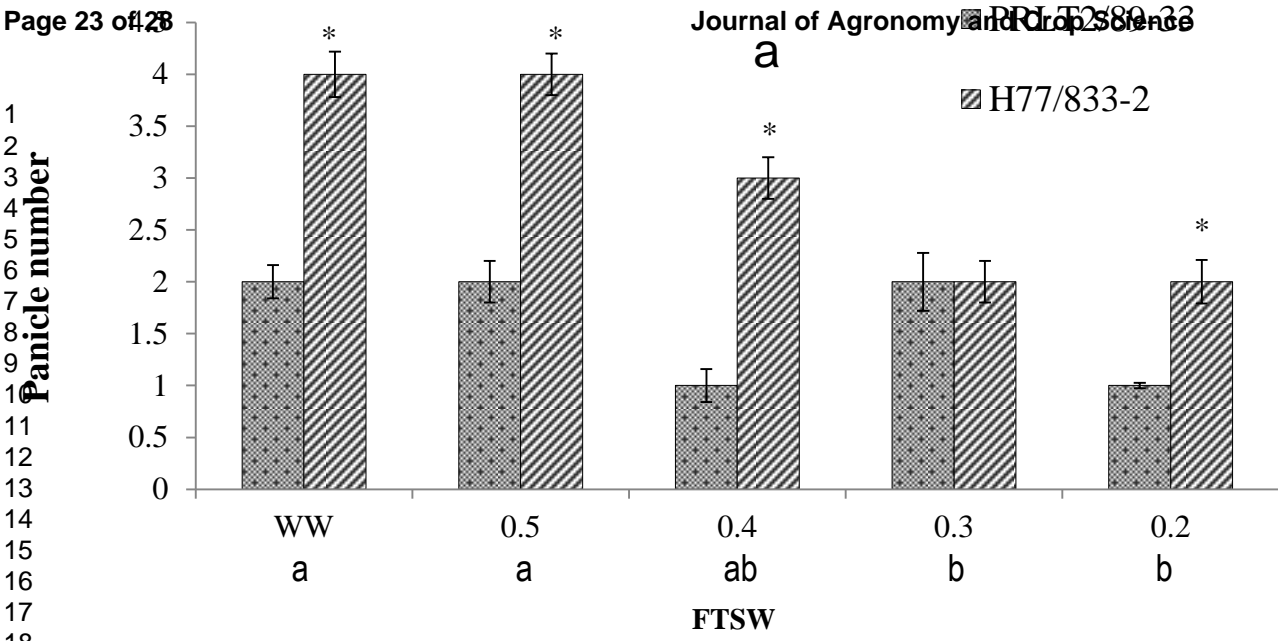


Figure 2

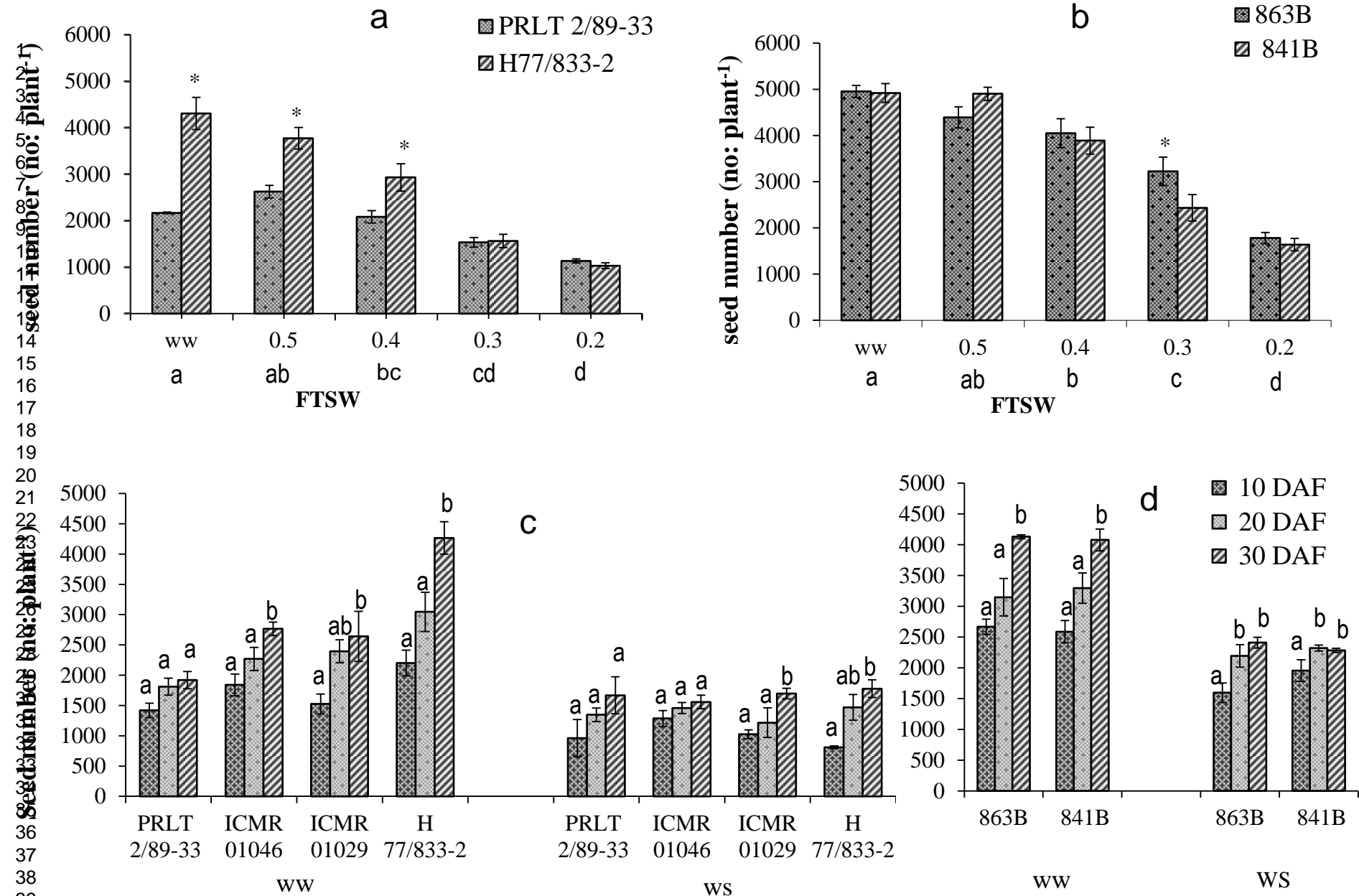


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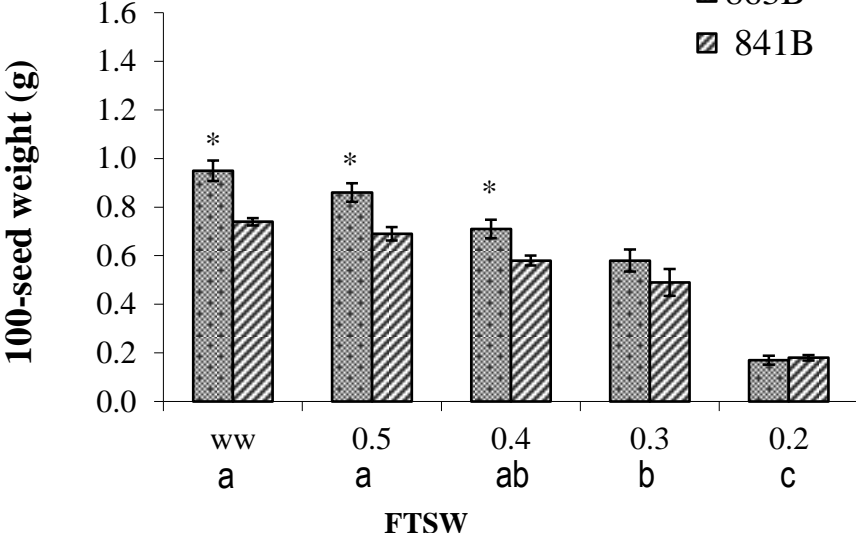
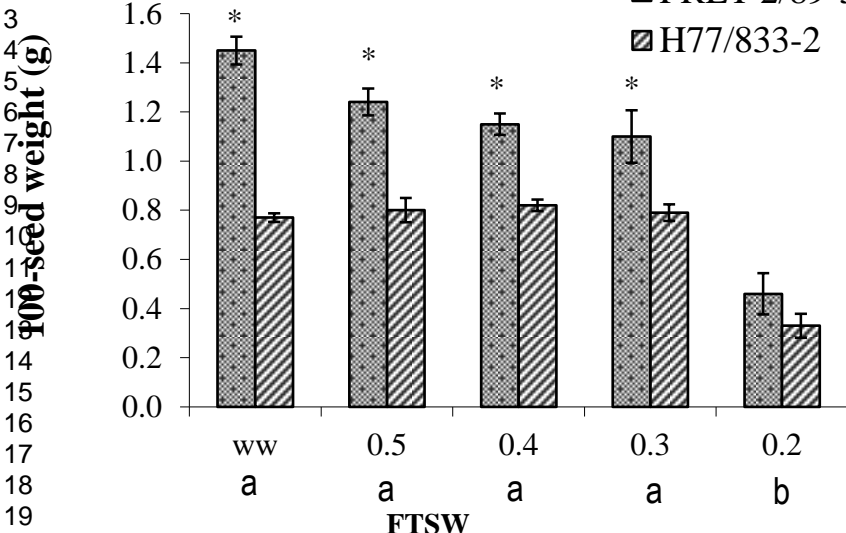
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■ PRLT 2/89-33  
▨ H77/833-2

■ 863B  
▨ 841B



c

d

■ 10 DAF  
▨ 20 DAF  
▨ 30 DAF

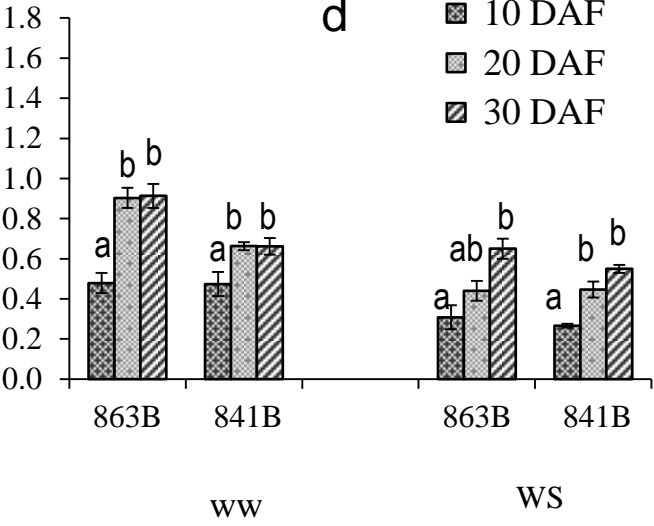
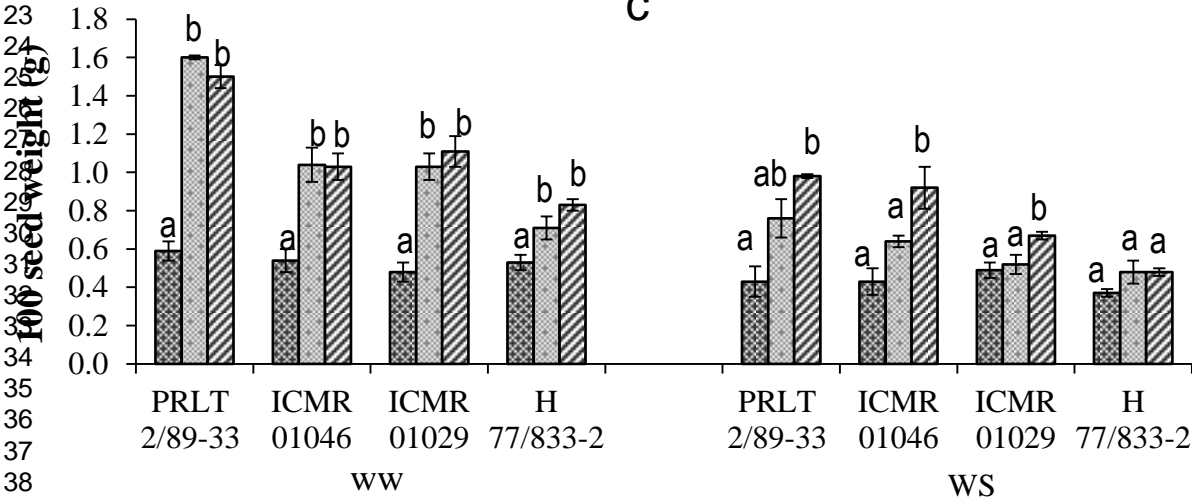


Figure 4

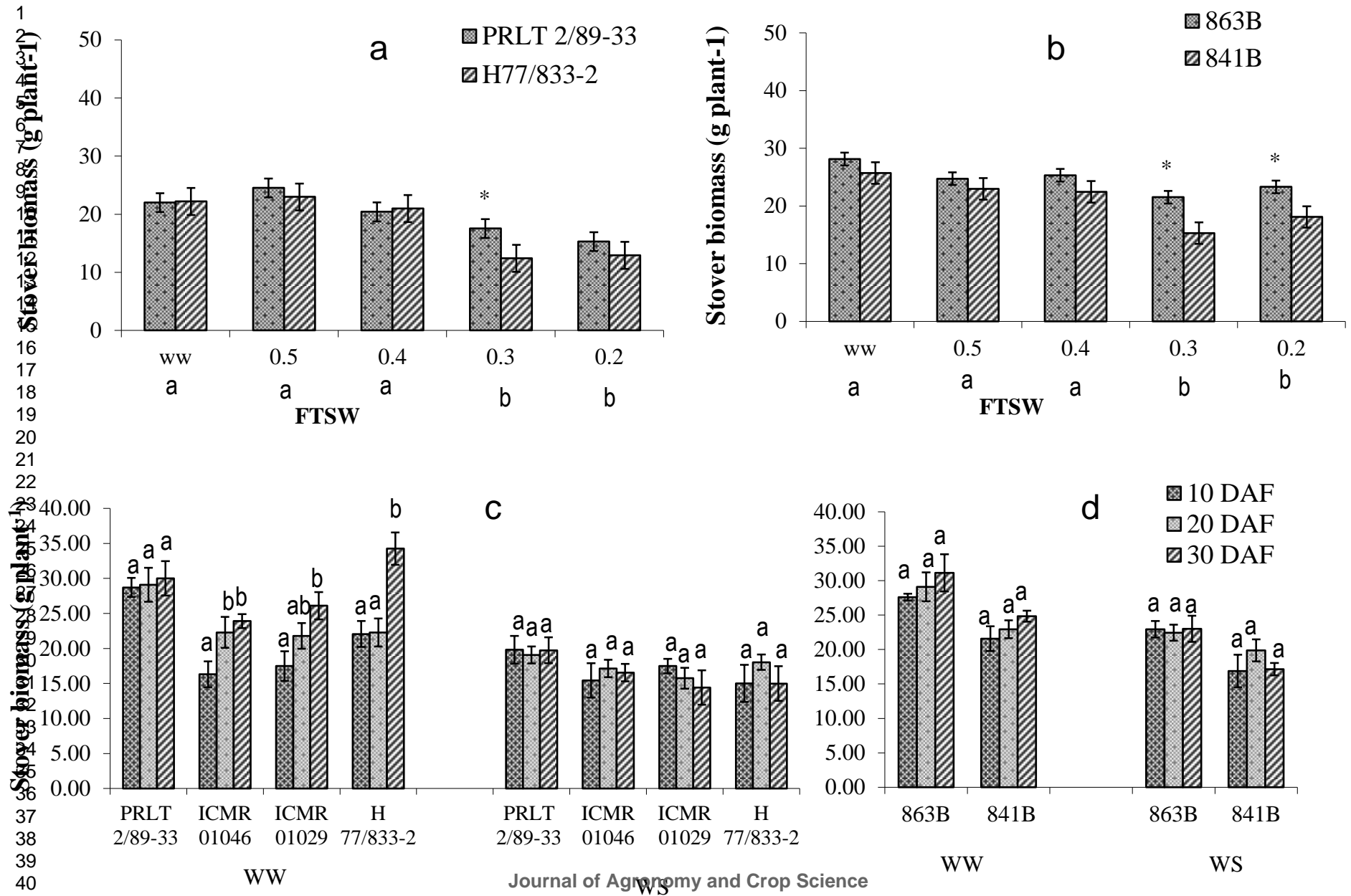


Figure 5

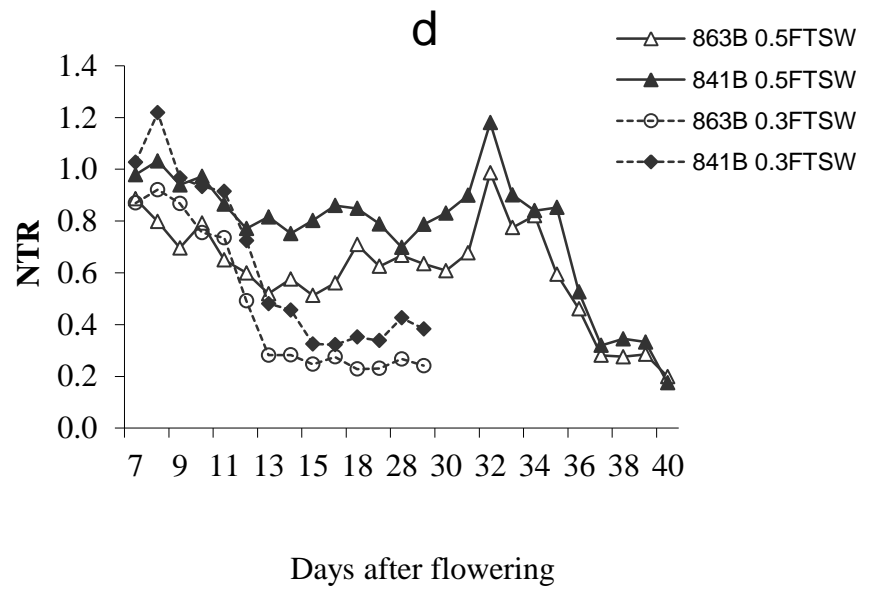
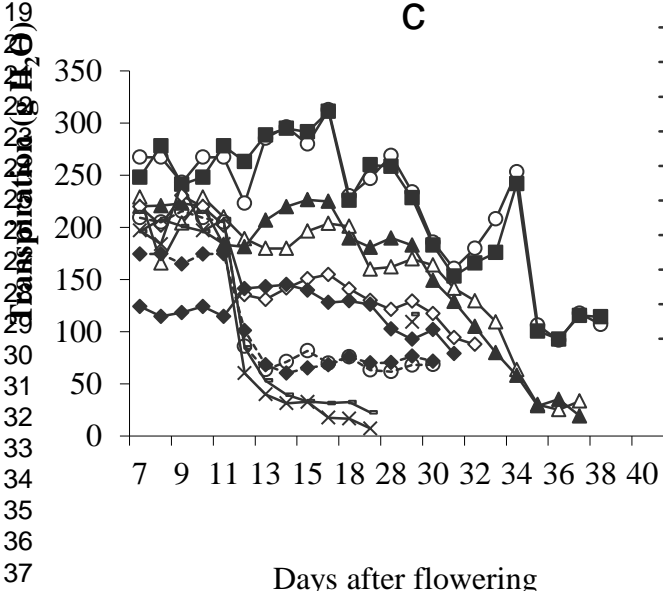
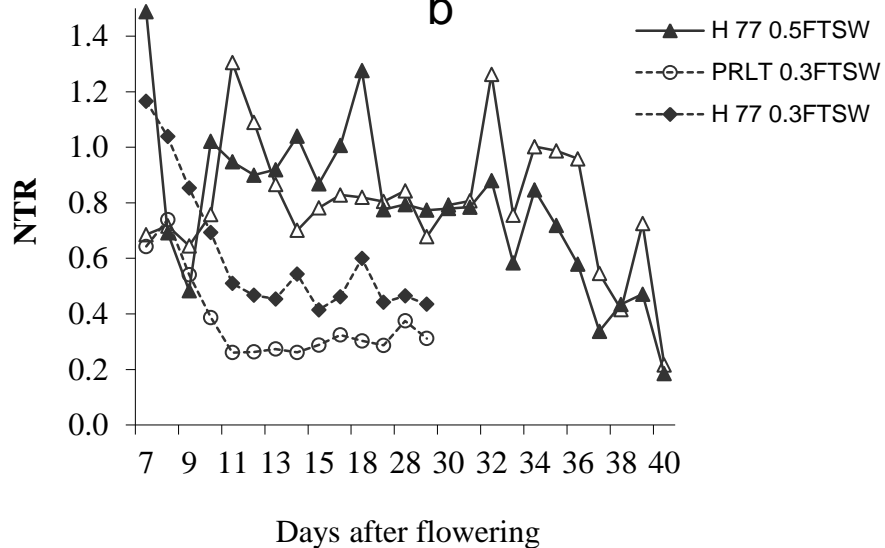
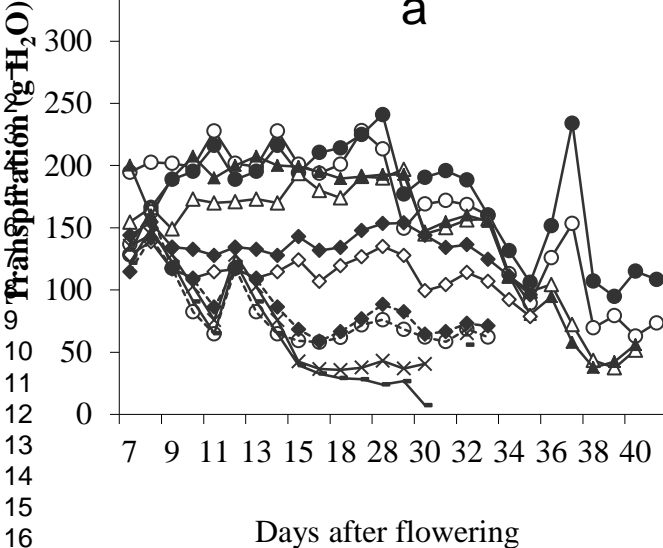


Figure 6



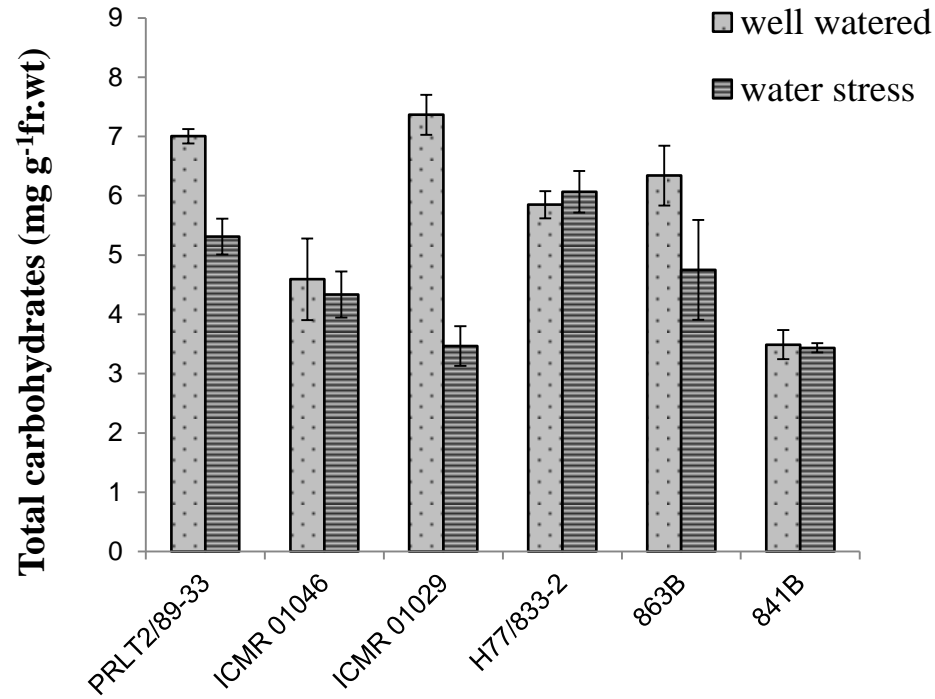


Figure 7